THE DETERMINATION OF SULFUR DEFICIENCY IN SOILS

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University of Alberta

April, 1959

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THE DETERMINATION OF SULFUR DEFICIENCY IN SOILS

A DISSERTATION

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF SOIL SCIENCE

by

DONALD ROBERT WALKER

EDMONTON, ALBERTA

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UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "The Determination of Sulfur Deficiency in Soils" submitted by Donald Robert Walker in partial fulfilment of the requirements for the degree of Master of Science.

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THE DETERMINATION OF SULFUR DEFICIENCY IN SOILS

ABSTRACT

Various laboratory analyses were done on alfalfa, alsike clover, red clover and soil samples from a number of field test sites in West-Central Alberta in an endeavor to find a laboratory means of establishing the available sulfur status of soils. Sodium sulfate was applied to the growing crop at each test site in order to determine responsiveness to sulfur fertilization. Yield increases, indicating sulfur deficient soils, were obtained at 72 of the 157 test locations.

Soluble sulfate, sulfate accumulation on incubation and the growth of Aspergillus niger were measured on thirty-six selected soil samples from the test sites. None of these determinations showed a relationship to the available sulfur supply of the soils as determined by plant growth response to sulfur fertilization.

The alfalfa and clover samples were analysed for total nitrogen, amide nitrogen, total sulfur, extractable sulfate and extractable sulfur. Amide nitrogen data were erratic and did not indicate the level of the sulfur nutrition of the plants. The extractable sulfate content of the alfalfa and alsike clover samples analysed differentiated the samples from sulfur deficient soils and the samples from non-sulfur deficient soils for 89 per cent of the alfalfa samples and 95 per cent of the alsike clover samples. This sulfur fraction did not differentiate the red clover samples. Extractable sulfur content was a reliable analysis for determining the need for sulfur fertilization for 89 per cent of the alfalfa samples, 100 per cent of the alsike clover samples and 88 per cent of the red clover samples.

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INTRODUCTION

The occurrence of sulfur deficient soils in West-Central Alberta has been recognized for some time (48). However, this is a large area and the exact distribution of sulfur deficiency has not been established. In an attempt to determine the extent and pattern of the sulfur deficiency, applications of sodium sulfate were made on legume crops. The tests covered a number of soil series on black, degraded black, grey wooded and peat soils. This survey showed that sulfur deficiency, as measured by legume yield response, follows neither a geographical nor a soil series pattern in the area under study. This conclusion agrees with the findings of Martin (39), who recently made a similar survey in California.

Since no proven method other than field testing is presently available for determining sulfur deficiency in soils, samples of plant and surface soil were collected from a number of the field plots and analysed in an endeavor to develop a laboratory means of determining the need for sulfur fertilization of legume crops.

LITERATURE REVIEW

Soil sulfur exists as both organic and inorganic compounds. The organic fraction, which represents generally from 50 to 90 per cent of the total soil sulfur, is present largely as the protein material from plant residues (19, 70). Other organic compounds, including taurine and mustard oils, may introduce very small amounts of sulfur into the soil (70). The inorganic sulfur fraction, accounting for 10 to 50 per cent of the total sulfur, is composed primarily of pyrite (FeS_2) with minor amounts of other mineral sulfides and varying quantities of gypsum ($CaSO_4.2H_2O$) and epsomite ($MgSO_4.7H_2O$) (34). Unless there is an appreciable amount of gypsum and/or epsomite present, most of the sulfur will be in an unoxidized state. The foregoing suggests that several approaches might be tried when seeking a determination for the sulfur fraction that is available to plants.

The total sulfur content of mineral soils of the humid temperate region varies from 0.01 to 0.15 per cent (34). Wyatt and Doughty (74) found the range of total sulfur for a number of Alberta soils to be 0.03 to 0.07 per cent. Bentley et al. (4) quoted a similar range of total sulfur in some Alberta soil samples collected from both locations that had and had not shown definite responsiveness to sulfur fertilization of legumes. These workers concluded that total soil sulfur was an unsuitable means of determining responsiveness to sulfur fertilization.

Sulfate soluble in various soil extractants has not proved to be a satisfactory criterion for measuring the available sulfur status of soils (12, 31). Saskatchewan data (59) and unpublished data from the Lacombe Experimental Farm have shown very pronounced yield responses of legumes from applications of sulfur fertilizers supplying as light a rate as

seven pounds of sulfur per acre. On the basis of a six-inch surface layer of soil weighing two million pounds, this rate of fertilization represents a concentration of 3.5 parts per million of sulfur. With such a small difference in the concentration of sulfur between sulfur deficient and non-sulfur deficient soils, and because of the difficulty of obtaining a soil extracting solution that will reproduce the extracting properties of plant roots, the extraction and measurement of soil sulfate would not seem to be very promising as a method for the determination of sulfur deficiencies.

The oxidation of organic and reduced inorganic sulfur compounds in soils has been studied in some detail (23, 38, 46, 54) but only to a limited extent as a measure of the available sulfur status of soils. Cairns (12), using the flask method of Fitts et al. (20) found that a soil on which alfalfa had responded to sulfur fertilization accumulated one part per million of sulfate-sulfur while a soil on which alfalfa had not responded to sulfur fertilization accumulated 15 parts per million sulfate-sulfur after two weeks' incubation.

Two biological or bioassay methods have been used for measuring available soil sulfur, microbial activity and the growth of Aspergillus niger.

Swaby (66) added glucose, nitrate, phosphate and calcium to a sample of soil and then determined the microbial activity of the sample in a macro-respirometer by making a continuous record of oxygen uptake and an intermittent record of carbon dioxide output. He compared this respiration curve with a similar curve obtained when sulfate was also added to the soil sample. With this technique gross deficiencies of sulfur could be detected.

Aspergillus niger requires sulfur for growth and when feeding on sugar rapidly uses sulfur present in the growth medium (70). Garreau (25) and Steinberg (64) found that alkyl sulfonate, alkyl sulfinate, cystine, homocystine, methionine, cysteine and taurine served as sources of sulfur for the organism while sulfides, disulfides, cysteic acid, ammonium ethylen-sulfonate, ammonium isothionate, ammonium sulfoacetate and ammonium ethane-sulfonate were not utilized. Picci (53), Malavolta and Galli (36) and Grigg (28) have used the Aspergillus niger method outlined by Nicholas (49) for determining the sulfur status of soils. Too few soils have so far been studied to arrive at any conclusion as to the suitability of the method.

The availability of soil sulfur for plants might also be studied by analysing the plant material grown on the soil. This might involve a measure of sulfur fractions or of other compounds that are affected by abnormal sulfur nutrition.

Mehlich (41) states that soil sulfur is absorbed by plants as the sulfate ion. A part of the absorbed sulfate is reduced to become a constituent of a number of organic sulfur compounds including cystine, methionine, thiamine, biotin, allyl sulfide, vinyl sulfide, mercaptans, glutathione and cysteine (16, 27, 43). Biswas and Sen (8) using radioactive sulfur found that more than twenty-five sulfur compounds were formed during one hour of sulfate uptake by ten-day-old pea plants. The greatest accumulation of sulfur was in the sulfur containing amino acids methionine, cystine, homocysteine and taurine with taurine being dominant. Walker et al. (71) concluded that much of the sulfate absorbed by rape (Brassica napus) was accumulated in the inorganic form once the plant requirements for protein and volatile oil synthesis had been met. Analyses of sulfate sulfur in clovers, cotton, alfalfa, beans, buckwheat and oats have indicated a higher concentration in plants receiving adequate sulfur than in plants not

receiving adequate sulfur (12, 18, 44, 47, 51). Peterson (52) concluded that sulfate-sulfur was more reliable as a measure of sulfur deficiency than was any other sulfur fraction in plants.

Other plant components affected by variations in the supply of sulfur are chlorophyll, various nitrogen fractions and the enzymes acting on carbohydrates (1, 33, 60). Amide nitrogen was studied more extensively than other nitrogen fractions, chlorophyll or the carbohydrate enzymes. Mertz et al. (42), Coleman (15) and Thomas et al. (67) found increased amounts of amide nitrogen in alfalfa and clover plants grown on media lacking adequate sulfur. Varner and Webster (69) showed that a large number of plants including members of Leguminosae contain an enzyme system for the formation of glutamine from glutamic acid and ammonia by the following reaction:

$$\text{Glutamate} + NH_3 + \text{ATP} \xrightarrow{Mg^{++}} \text{glutamine} + \text{ADP} + P_i \quad (1)$$

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(1) Abbreviations used: ATP - adenosine triphosphate; ADP - adenosine diphosphate; P_i - inorganic phosphate.

MATERIAL AND METHODS

Field Plots

Sodium sulfate was applied at eighty pounds per acre (20 lb./ac. S) to a four square rod plot on 157 locations in 1957 and 1958. The area covered by these plots is shown on the map of West-Central Alberta in Figure 1. Soil samples of the 0-6 inch surface layer were taken from most locations. Botanically pure plant samples, consisting of whole tops of plants, were gathered from a number of the test areas where sulfur deficiency was evident in 1957. These samples were taken from both the fertilized plot area and the adjacent unfertilized area. In 1958 plant samples were collected from both sulfur deficient and non-sulfur deficient test areas.

The 1957 plant samples were collected in late July in most cases while a few samples were collected from the second growth in early September. The 1958 samples were collected in early July. The samples were hung in jute bags inside an unheated building for from two to eight days and they were then dried in a forced air dryer at 205°F. The samples were ground for analysis after drying.

Analytical Methods

Plant Material

Numerous extracting solutions have been used for extracting sulfate and amides including hot 25% HCl, cold H_2O, hot H_2O, cold 5% trichloroacetic acid, hot 5% trichloroacetic acid, 80% ethyl alcohol, 60% ethyl alcohol and 87% ethyl alcohol (3, 10, 11, 30, 51, 57, 73). Two extractions with boiling 70% ethyl alcohol used in the ratio 25:1 extractant to plant material were

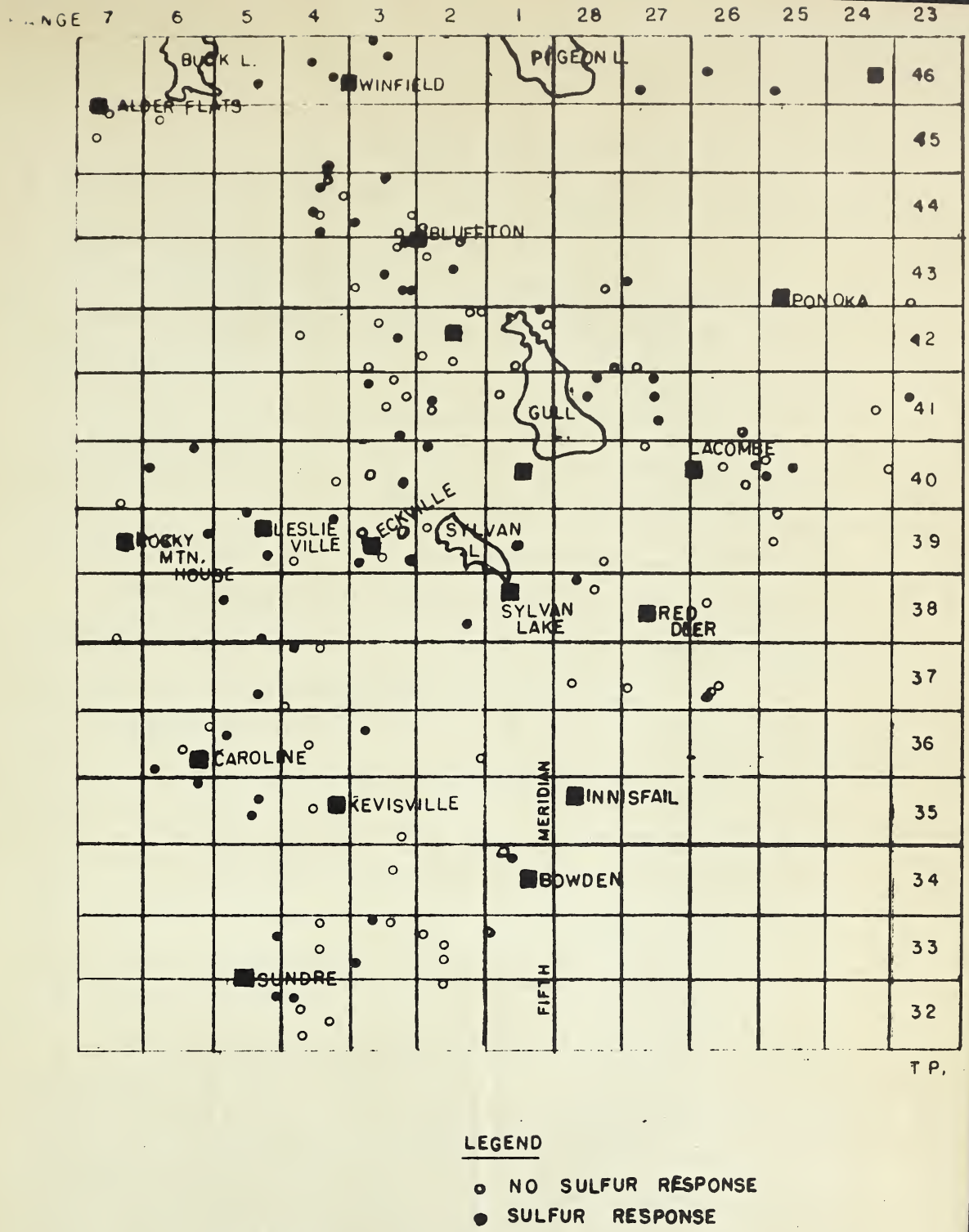

Fig. 1. Map of West-Central Alberta showing location of sulfur test plots 1957-58

found to give good removal of sulfate and amide nitrogen while at the same time effectively precipitating the protein and that method was used in this study.

A three-gram sample of air dry plant material was extracted twice in a Waring blendor using 75 ml. of hot 70% ethyl alcohol for each extraction. Each extraction was for ten minutes. The mixture was filtered through a Buchner funnel using suction, the filter paper from the first extraction being returned to the blendor for the second extraction. The combined filtrates were made up to a volume of 250 ml. with distilled water and three 2 ml. aliquots were removed for amide nitrogen determination.

The remaining solution was evaporated to dryness on a hot plate and treated for the determination of either the sulfate soluble in hot 70% ethyl alcohol or the sulfur soluble in hot 70% ethyl alcohol.

For the determination of the sulfate soluble in hot 70% ethyl alcohol, 50 ml. of 0.1 N hydrochloric acid were added to dissolve the material in the beaker. One heaping tablespoon of Norit A activated charcoal was stirred in and the sample was allowed to stand for fifteen minutes before filtering through a Buchner funnel using suction. The filtrate was then made up to 250 ml. with 0.1 N hydrochloric acid and saved for sulfate analysis. This sulfur fraction is hereafter referred to as extractable sulfate.

For the determination of sulfur soluble in hot 70% ethyl alcohol, the extract, after being taken to dryness, was treated with 15 ml. of concentrated nitric acid and 9 ml. of 70% perchloric acid and again evaporated to dryness. Any non-sulfate sulfur in the sample was thereby converted to sulfate. The cleared sample was then made up to a volume of 250 ml. with 0.1 N hydrochloric acid and saved for sulfate analysis. This sulfur fraction is hereafter referred to as extractable sulfur.

Sulfate can be determined gravimetrically, volumetrically, turbidimetrically, electrometrically or colorimetrically (2, 4, 5, 7, 8, 9, 13, 14, 17, 21, 24, 29, 32, 35, 37, 56, 61, 63, 65). The volumetric methods of Sporek (63) and Ashgar et al. (2) were tried but the amount of sulfate present in the plant material was insufficient for accurate volumetric determination. The turbidimetric method of Bentley et al. (4) was therefore adopted, the readings being made with a Beckman model B spectrophotometer using a wavelength seting of 375 mμ, a sensitivity setting of 4 and a slit width of 0.02. This procedure proved satisfactory for sulfate in the concentration range of 0 to 50 parts per million.

The three aliquots saved for amide nitrogen determination were distilled according to the method of Varner et al. (68) without the preliminary distillation to remove ammonia. Two tests were conducted to determine the suitability of this procedure which is much simpler than the photometric ninhydrin method of Moore and Stein (45) or the hydrolysis methods used by McKee (40) and Pucher et al. (55). The procedure of Varner et al. (68) was used to test two samples of each legume for the presence of ammonium nitrogen. The results, shown in Appendix I, were negative, any ammonium nitrogen that had been present in the samples probably being expelled during the oven drying of the plant material. Recovery of aspargine and glutamine was tested using water solutions of known concentrations of the two amides. As reported in Appendix II, recovery ranged from 91 to 97 per cent. The recovery was considered satisfactory for this study since fairly substantial differences in amide content were required if the determination was to be used as a measure of the level of the sulfur nutrition of the plants.

Total sulfur content of the plant material was determined by using the nitric-perchloric acids oxidation procedure of Gieseking et al. (26)

and determining the sulfur turbidimetrically.

Total nitrogen was determined by a micro-kjeldahl procedure.

Soil Samples

Numerous extractants have been proposed for removing soil sulfate. Freney (22) and Little (35) used water while Ensminger (17) found that water extracted practically no sulfate. He found that neutral sodium acetate, sodium acetate buffered at pH 4.8 and potassium dihydrogen phosphate solution containing either 50 or 100 parts per million of phosphorus extracted similar amounts of sulfate from the soils tested. Other weakly acidic extractants have also been tested (12, 14, 21, 56).

Because of the inconclusive nature of the results obtained with the various sulfate extractants which have been tested elsewhere, three extractants were tested on four of the soil samples from this study. Tenth normal hydrochloric acid, 0.001 N hydrochloric acid and 70% ethyl alcohol were tested, a ratio of 2:1 of extractant to soil being used. The data, presented in Appendix III, show that 0.1 N hydrochloric acid and 70% ethyl alcohol extracted similar quantities of sulfate in three of the four soils while 0.001 N hydrochloric acid extracted considerably more. With the fourth soil 0.1 N and 0.001 N hydrochloric acid extracted approximately twenty times more sulfate than did the 70% ethyl alcohol. The two soils from which 0.001 N hydrochloric acid extracted 5 and 6 parts per million of sulfate were sulfur deficient as indicated by legume response to sulfur fertilization. The other two soils did not show sulfur deficiency and the amount of sulfate extracted from these soils by the 0.001 N hydrochloric acid extractant was 10 parts per million for one soil and 35 parts per million for the other soil. For this reason 0.001 N hydrochloric acid was used for the sulfate extractions.

For soil sulfate determination, one hundred grams of soil were shaken with 200 ml. of extractant for one hour. The sample was filtered through a Buchner funnel using a filter paper and a layer of celite. The filtrate was taken to dryness on a hot plate and color was removed. Activated charcoal was compared with nitric acid - perchloric acid oxidation for clarifying the filtrates. The results, shown in Appendix III, indicated that some non-sulfate sulfur was extracted by the 0.001 N hydrochloric acid. Clarification was, therefore, accomplished with Norit A charcoal, one teaspoon being added with 15 ml. of 0.1 N hydrochloric acid. This solution was filtered through a Buchner funnel using a filter paper and a thin layer of celite. The filtrate was diluted to 25 ml. with 0.1 N hydrochloric acid and the sulfur was determined turbidimetrically.

The procedure for determining sulfate accumulation in incubated soils utilized 100 gm. soil samples mixed with fifty cubic centimeters of perlite[1)] in 375 ml. shaker jars. The perlite was added to aid moisture retention and improve aeration. Moisture levels varying from 15 to 20 per cent in soils with a sand to clay ratio of 3:1, to 60 cm. tension in loam soils and 30 cm. tension in loamy sand, silt loam and silty clay have been recommended for incubation for sulfur oxidation in soils (46, 54). In these tests a uniform level of moisture percentage was adopted, 25 ml. being added to each sample. The bottles were capped with a one-hole stopper and incubated at 30°C. for a two-week period. Sulfate was then extracted and determined as previously outlined.

Bioassay for available soil sulfur using Aspergillus niger was done according to the method of Picci (53) outlined by Malavolta et al. (36).

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1)Mfged. by Great Lakes Carbon Corp., Perlite Div., Linden, New Jersey.

Nicholas (49) using Aspergillus niger for the detection of minor mineral deficiencies found Mulder's strain of the organism to be the most satisfactory one tested since no sporulation occurred if iron, zinc or molybdenum were absent whereas some sporulation invariably occurred with the Steinberg strain. Mulder's strain[1)] was, therefore, used in this bioassay.

[1)] Obtained from Horticultural Div., Central Experimental Farm, Ottawa.

RESULTS AND DISCUSSION

Growth response to applied sulfur

In 1957 visual response ratings were made for all field test plots early in July and in addition a number of the plots showing yield increases were sampled. A fifty square foot sample was cut from the sulfur fertilized plot and also from the adjacent unfertilized area. The yield data from the 1957 harvested plots are shown in Table 1. The data in Table 1 show generally a very good response to applied sulfur fertilizer. The very low yields obtained at several locations were due to the extremely dry conditions that occurred in parts of West-Central Alberta in 1957. Tests number 33, 60 and 62 which had not yielded heavily in 1957 showed very good growth and response to sulfur fertilizer in 1958 when moisture conditions were better. The yield data include all growth on the sampled areas and wherever a very thin stand of legumes was encountered, the data are of dubious value since weed growth was much heavier on the unfertilized plots than on the fertilized plots. This difference in weed growth was the result of greater competition from the legumes supplied with adequate sulfur.

The 1958 test plots were rated visually and yield increases estimated. With the exception of three locations that showed color differences only, the range of increase for plots showing response was from 25 to 500 per cent over the unfertilized plots.

For the two years 1957 and 1958, yield increases, indicating sulfur deficient soils, were obtained at 72 of the 157 test locations.

Table 1 - Yield data from 1957 field tests

Test No.	Location	Legume present	Yield, tons/ac. D.M. No fertilizer	Yield, tons/ac. D.M. S* at 20 lb./ac.
1	SE22-35-5-W5	Alsike	0.42	0.69
4	SE32-41-3-W5	"	0.08	0.47
31	NE 2-37-5-W5	"	0.23	0.42
33	NW36-40-6-W5	"	0.20	0.68
38	NE 2-37-5-W5	"	0.37	0.99
60	NW34-39-5-W5	"	0.20	0.37
62	NW34-39-5-W5	"	0.14	0.17
184	SW15-44-4-W5	"	0.44	2.27
185	NE28-46-3-W5	"	1.40	2.62
187	SE28-43-2-W5	"	0.32	0.65
188	NW15-43-3-W5	"	1.99	2.99
189	SW13-43-3-W5	"	0.60	1.56
20	SE25-33-5-W5	Red clover	0.29	0.33
36	NW20-41-2-W5	" "	0.50	1.30
93	NE35-43-3-W5	" "	1.13	1.56
183	SW33-34-1-W5	Alfalfa	0.47	1.03
17	SW29-36-5-W5	Mixtures	0.33	1.24
18	NW19-38-5-W5	"	0.21	0.43
25	SE31-32-4-W5	"	0.85	1.22
57	NE19-38-5-W5	"	0.19	0.69
58	NW 5-36-6-W5	"	0.56	1.24
186	NW 7-44-3-W5	"	1.08	1.37

*S applied as Na_2SO_4.

Analytical Results

Plant Material

Sulfur fractions

Data for total sulfur, extractable sulfate and extractable sulfur are presented in Tables 2, 3 and 4 for the various crops.

Table 2 - The effect of fertilization on the sulfur content of alfalfa

Test No.	Location	Year of Test	No sulfur applied			20 lb./ac. sulfur applied		
			% Total S	Extractable Sulfate %	Extractable* Sulfur %	% Total S	Extractable Sulfate %	Extractable* Sulfur %
			Non-sulfur deficient test areas					
100	NE18-39-25-W4	1958	0.24	0.21	0.30	0.24		0.34
64	NW32-39-25-W4	1958	0.17	0.07	0.17	0.28		0.39
137	NW14-39- 1-W5	1958	0.26	0.23	0.35	0.28		0.38
128	NW16-37-28-W4	1958	0.24	0.18	0.22	0.22		0.24
83	SW 9-37-26-W4	1958	0.28	0.28	0.36	0.24		0.30
84	NE 9-37-26-W4	1958	0.18	0.05	0.13	0.24		0.37
121	SW18-37-27-W4	1958	0.24	0.18	0.30	0.26		0.28
65	NE25-41- 3-W5	1958	0.27	0.25	0.39	0.28		0.38
70	NW36-42- 1-W5	1958	0.20	0.16	0.31	0.23	0.12	0.26
171	NE10-44- 4-W5	1958	0.16	0.04	0.10	0.37		0.74
131	SW 9-37-26-W4	1958	0.28	0.28	0.49	0.32	0.20	0.61
			Sulfur deficient test areas					
45	NW17-46-26-W4	1957	0.19	0.05	0.18	0.26		0.26
9	SW 8-46-25-W4	1957	0.17	0.09	0.22	0.34		0.26
42	SW 7-33- 3-W5	1957	0.16	0.02	0.08	0.33		0.26
54	SW36-39- 4-W5	1957	0.13	0.05	0.05	0.17		0.07
53	SW33-38-28-W4	1957	0.17	0.03	0.10	0.29		0.21
24	NE32-40- 2-W5	1957	0.16	0.04	0.04	0.25		0.24
32	SE16-39-28-W4	1957	0.20	0.05	0.17	0.31		0.30
30	NW33-33- 3-W5	1957	0.14	0.03	0.10	0.20		0.22
68	NE32-40- 2-W5	1958**	0.16	0.02	0.09	0.18		0.20
142	SE27-41-28-W4	1958	0.17	0.03	0.06	0.24		0.26
97	NW18-40-25-W4	1958	0.16	0.02	0.10	0.20		0.27
91	NE24-40-26-W4	1958	0.12	0.02	0.09	0.22		0.26
94	SW23-46- 4-W5	1958	0.22	0.06	0.17	0.36	0.25	0.39
104	NE33-44- 3-W5	1958	0.11	0.02	0.08	0.21		0.24
117	NE 6-39- 3-W5	1958	0.11	0.02	0.02	0.51		0.60
123	NE35-41-28-W4	1958	0.18	0.05	0.13	0.17		0.17
154	NW20-41-23-W4	1958	0.12	0.01	0.08	0.21	0.10	0.17
151	NE20-41-27-W4	1958	0.19	0.07	0.12	0.28		0.36

*Expressed as sulfate.
**Residual effect of sulfur applied in spring 1957 - same plot as test No. 24.

Table 3 - The effect of available sulfur level on the sulfur content of Alsike clover

Test No.	Location	Year of Test	No sulfur applied			20 lb./ac. sulfur applied		
			% Total S	Extractable sulfate %	Extractable* sulfur %	% Total S	Extractable sulfate %	Extractable* sulfur %
			Non-sulfur deficient test areas					
69	SE 1-42-28-W5	1958	0.16	0.02	0.09	0.21		0.18
82	NW12-44- 3-W5	1958	0.17	0.07	0.11	0.23	0.14	0.27
90	NW27-38-28-W4	1958	0.21	0.13	0.24	0.20		0.17
132	SE-32-41- 3-W5	1958	0.17	0.05	0.13			
133	NE35-44- 4-W5	1958	0.19	0.10	0.18	0.24		0.29
150	SE20-39- 3-W5	1958	0.19	0.05	0.14	0.23	0.10	0.20
			Sulfur deficient test areas					
4	SE32-41- 3-W5	1957	0.10	0.02	0.03	0.14		0.15
31	NE 2-37- 5-W5	1957	0.12	0.02	0.01	0.19		0.12
34	NE19-40- 6-W5	1957	0.11	0.02	0.01	0.18		0.06
38	NE 2-37- 5-W5	1957	0.10	0.04	0.03	0.14		0.08
60	NW34-39- 5-W5	1957	0.09	0.02	0.05	0.16		0.13
61	SW13-40- 3-W5	1957	0.09		0.06	0.14		0.07
62	NW34-39- 5-W5	1957	0.08	0.02	0.01	0.12		0.08
80	NE21-40-25-W4	1958	0.13	0.02	0.04	0.20		0.18
81	SE 8-41-27-W4	1958-	0.13	0.02	0.07	0.21	0.07	0.18
88	SW15-46- 5-W4	1958	0.11	0.01	0.07			
103	NE19-40- 6-W5	1958	0.15	0.02	0.04	0.24	0.12	0.23
116	SW32-37- 4-W5	1958	0.10	0.02	0.01	0.24		0.17
120	NW26-44- 4-W5	1958	0.14	0.02	0.01	0.26		0.36
129	NW33-39- 5-W5	1958	0.14	0.02	0.03	0.36		0.63
156	SE24-39- 6-W5	1958	0.14	0.02	0.05	0.26		0.31
174	NE33-44- 3-W5	1958	0.12	0.02	0.01	0.24		0.23

*Expressed as sulfate.

Table 4 - The effect of available sulfur level on the sulfur content of red clover

Test No.	Location	Year of Test	No sulfur applied			20 lb./ac. sulfur applied		
			% Total S	Extractable sulfate %	Extractable* sulfur %	% Total S	Extractable sulfate %	Extractable* sulfur %
			Non-sulfur deficient test areas					
73	SE 3-44-3-W5	1958	0.17	0.04	0.09	0.18	0.03	0.10
92	SE30-39-2-W5	1958	0.18	0.02	0.09	0.19		0.10
115	SE34-43-2-W5	1958	0.15	0.03	0.10	0.17		0.13
130	NE24-44-4-W5	1958	0.13	0.03	0.06	0.24		0.20
134	NW11-38-2-W5	1958	0.14	0.02	0.05	0.17		0.11
149	NW29-45-6-W5	1958	0.20	0.03	0.06	0.21		0.16
159	NE30-43-2-W5	1958	0.19	0.05	0.09	0.18	0.05	0.12
160	SW20-41-2-W5	1958	0.19	0.05	0.12	0.21		0.15
163	SW33-45-7-W5	1958	0.18	0.03	0.09	0.20		0.15
			Sulfur deficient test areas					
1	SE22-35-5-W5	1957	0.12	0.04	0.05	0.15		0.09
17	SW29-36-5-W5	1957	0.08	0.03	0.01	0.13		0.10
18	NE19-38-5-W5	1957	0.10	0.03	0.04	0.14		0.12
20	SE25-33-5-W5	1957	0.11	0.03	0.02	0.16		0.12
36	NW20-41-2-W5	1957	0.07	0.03	0.03	0.12		0.11
57	NE19-38-5-W5	1957	0.06	0.02	0.02	0.09		0.05
58	NW 5-36-6-W5	1957	0.11	0.03	0.06	0.17		0.16
74	NE22-46-4-W5	1958	0.10	0.02	0.03	0.19		0.13
75	NW36-40-6-W5	1958	0.09	0.02	0.02	0.15	0.04	0.08
78	SE 3-44-4-W5	1958	0.13	0.02	0.06	0.22		0.18
87	SE13-43-3-W5	1958	0.09	0.03	0.02	0.21	0.10	0.21
93	NE35-43-3-W5	1958	0.11	0.02	0.02	0.20		0.15
107	NW31-40-2-W5	1958	0.15	0.03	0.07	0.19		0.13
114	SE 2-45-4-W5	1958	0.11	0.02	0.06	0.18		0.13
122	NW22-46-4-W5	1958	0.10	0.01	0.03	0.21		0.18
155**	SE 2-38-5-W5	1958	0.12	0.02	0.06	0.15		0.08
161	SW 1-41-3-W5	1958	0.13	0.05	0.08	0.24		0.22

*Expressed as sulfate.

**1957 application of sulfur.

Generally, the three crops contained a greater amount of total sulfur when grown on soils supplying adequate sulfur than when grown on soils not supplying adequate sulfur. The average total sulfur content of unfertilized plants was 0.23% and 0.16% for alfalfa, 0.18% and 0.12% for alsike clover and 0.17% and 0.10% for red clover when grown on non-sulfur deficient and sulfur deficient soils respectively. The data for total sulfur content of unfertilized plant samples on individual test locations are depicted in Figure 2.

Extractable sulfate was determined on all the non-sulfur treated samples and on four sulfur fertilized samples of each legume. Two of these latter samples were from sulfur deficient soils and two from non-sulfur deficient soils. The extractable sulfate data are shown in Figure 3. The data for alfalfa and alsike clover show consistent differences between samples from sulfur deficient and non-sulfur deficient soils with few exceptions. The lowest value determined for extractable sulfate for both alfalfa and alsike clover grown on non-sulfur deficient soil was for material from a location where a definite color difference between the sulfur fertilized and untreated plots was evident during the growing season. The greatest content of extractable sulfate of non-sulfur fertilized plants, grown at locations where yield response to sulfur fertilization was evident, should be a realistic value for separating soils which are deficient in sulfur from soils which are not deficient in sulfur. Using this criterion, the separating or critical level for alfalfa samples is 0.09 per cent. Three samples from non-sulfur deficient soils contained less extractable sulfate than 0.09 per cent and the reliability of this determination on the basis of the alfalfa samples analysed is approximately 89 per cent since there were 29 locations

Figure 2. Percentage total sulfur in non-sulfur fertilized legumes grown on sulfur deficient and non-sulfur deficient soils.

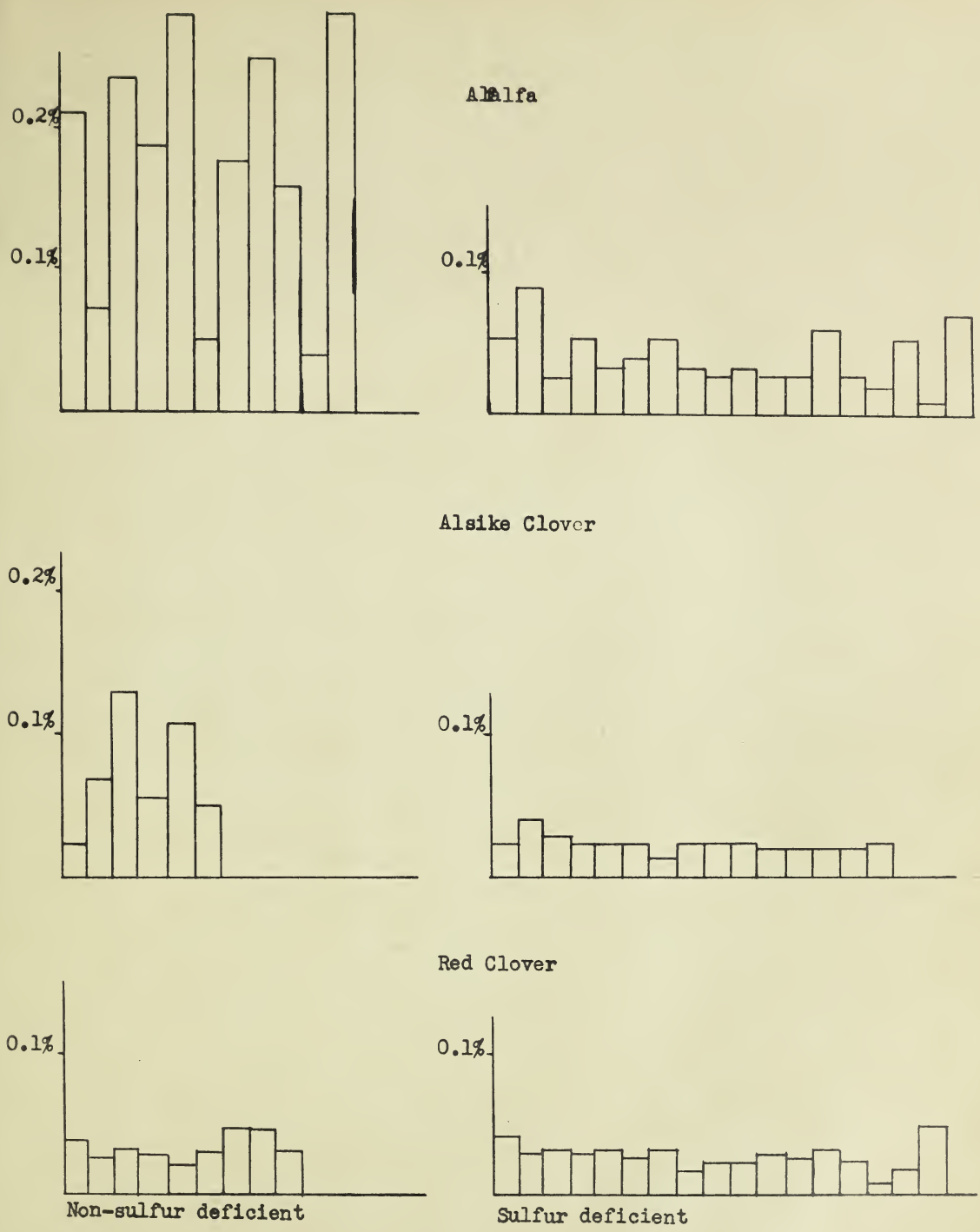


Figure 3. Percentage extractable sulfate in non-sulfur fertilized legumes grown on sulfur deficient and non-sulfur deficient soils.

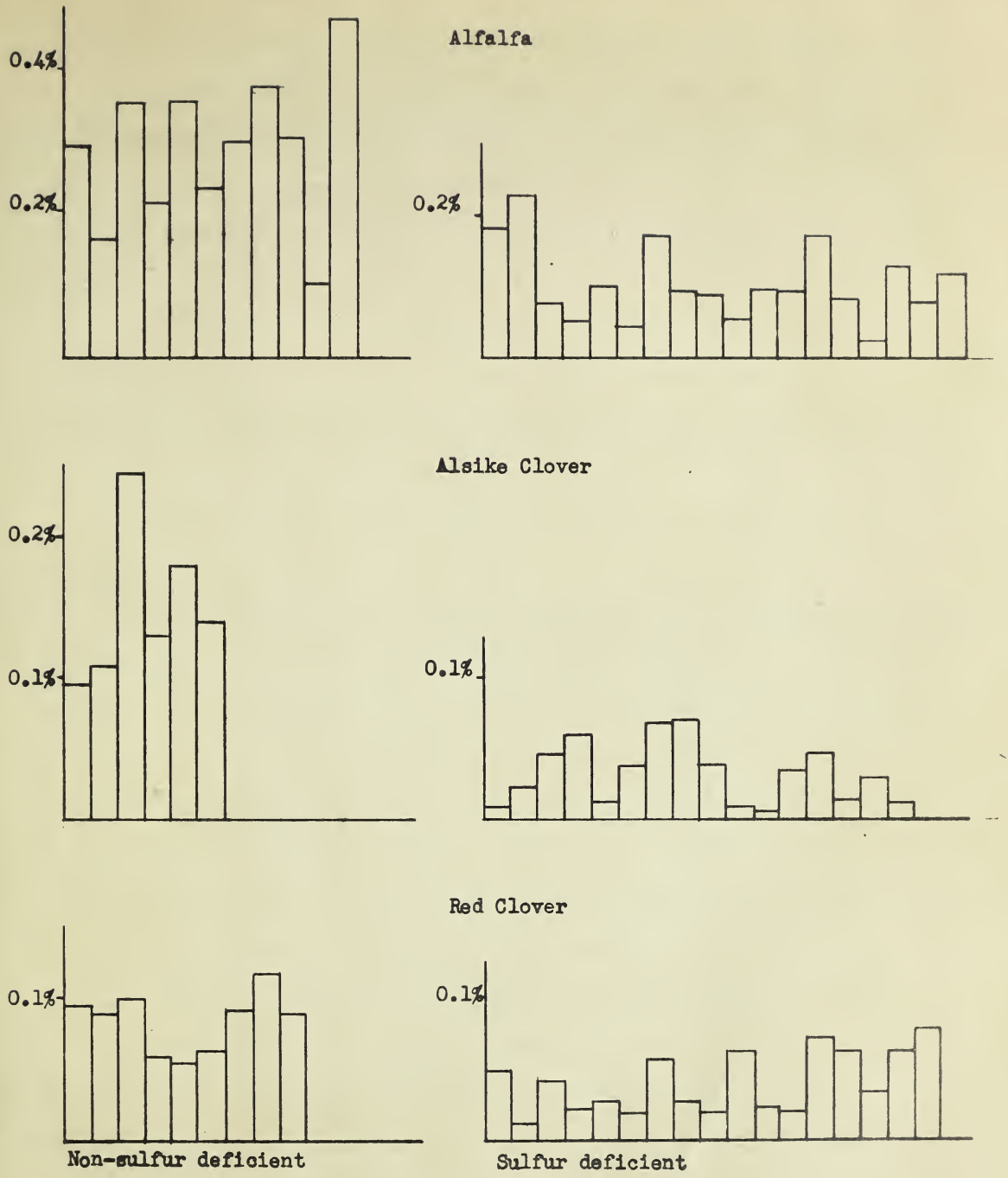


Figure 4. Percentage extractable sulfur in non-sulfur fertilized legumes grown on sulfur deficient and non-sulfur deficient soils.

from which alfalfa samples were procured and analysed. The critical level for alsike clover is 0.04 per cent extractable sulfate and this determination is reliable for 95 per cent of the tests. Extractable sulfate of red clover was an unsatisfactory measure since the highest value obtained for samples from sulfur deficient soils was as great or greater than the values for all samples grown on non-sulfur deficient soils.

The extractable sulfur content data for plant samples from unfertilized plots are presented in Figure 4. The critical level of extractable sulfur in alfalfa, using the criterion established for extractable sulfate, is 0.21 per cent. This determination is reliable for 89 per cent of the tests. The critical level for alsike clover is 0.07 per cent extractable sulfur, the determination being reliable in all cases.

The extractable sulfur content of red clover is a suitable determination for predicting the need for sulfur fertilization. The critical content of extractable sulfur is 0.08 per cent, this value being reliable for 88 per cent of the tests. However, eight of the nine red clover samples from non-sulfur deficient soil locations contain 0.10 per cent or less extractable sulfur. There is, then, a very narrow range of differences in values of extractable sulfur in plants from sulfur deficient soils and plants from non-sulfur deficient soils. The possibility of increasing the magnitude of this difference was considered. Worth and Krishnan (72) and Nightingale (50) found that alfalfa and tomato plants accumulated a higher concentration of sulfate in the leaves than in the stems. Since higher concentrations of sulfate might also result in a greater magnitude of difference in content of plants receiving an adequate sulfur supply, leaf and leaf plus stem samples of alfalfa and red clover were analysed for extractable sulfate and extractable sulfur. The results, given in Appendix IV, reveal that alfalfa leaves contain appreciably more extractable sulfate and extractable sulfur than the stems, but there is no appreciable

difference in the content of either sulfur fraction between the leaves and stems of red clover. This method of attempting to increase the magnitude of differences in content of the two sulfur fractions in red clover was, therefore, discarded.

Total nitrogen percentages and amide nitrogen content data for the three legumes are tabulated in Tables 5, 6 and 7.

Total nitrogen data were averaged for sulfur deficient and non-sulfur deficient test areas for each legume and these data are presented in Table 8. The red clover samples from non-sulfur deficient soils contained considerably more total nitrogen than did the samples grown on sulfur deficient soils. The alfalfa samples contained the same amount of total nitrogen whether grown on sulfur deficient or non-sulfur deficient soils. Since the total nitrogen values presented in Table 8 are averages, the small difference noted for alsike clover grown on sulfur deficient and non-sulfur deficient soils is unlikely to be a true difference. The application of sulfate fertilizer has increased the total nitrogen content of the three legumes on both groups of soils. The variation in total nitrogen content due to seasons is indeed interesting. Alfalfa had a higher total nitrogen content in 1957 than in 1958 while the reverse was true for alsike and red clovers. The seasonal variations approach the magnitude of the differences due to sulfur fertilization of the three legumes.

The amide nitrogen data, presented in Tables 5, 6 and 7, are very erratic for the three legume crops. This might possibly be due to the delay period of two to eight days between harvesting and drying the samples. Some respiration would take place and since there would be no synthesis of carbohydrate some respiration of protein, with a resultant accumulation of amide, could occur.

Table 5 - Effect of sulfur supply on the amide nitrogen and total nitrogen content of alfalfa

Test No.	Location	Year of Test	No sulfur applied			20 lb./ac. sulfur applied		
			% total N	Amide N		% total N	Amide N	
				mgm./gm. plant material	mgm./gm. total N		mgm./gm. plant material	mgm./gm. total N
			Non-sulfur deficient test areas					
100	NE18-39-25-W4	1958	2.75	1.0	38	2.68	1.2	44
64	NW32-39-25-W4	1958	2.96	2.0	67	2.99	2.0	67
137	NW14-39- 1-W5	1958	3.10	1.5	49	3.40	1.7	49
128	NW16-37-28-W4	1958	3.36	1.4	41	3.10	1.7	54
83	SW 9-37-26-W4	1958	2.71	0.6	23	2.41	0.8	34
84	NE 9-37-26-W4	1958	2.79	1.4	48	2.96	1.5	52
121	SW18-37-27-W4	1958	2.85	1.4	51	2.94	1.8	61
65	NE25-41- 3-W5	1958	3.03	1.8	58	3.20	0.5	16
70	NW36-42- 1-W5	1958	2.98	1.8	61	2.88	1.4	48
171	NE10-44- 4-W5	1958	3.26	2.0	62	3.45	1.3	38
131	SW 9-37-26-W4	1958	2.94	1.2	40	3.13	1.3	42
			Sulfur deficient test areas					
45	NW17-46-26-W4	1957	3.57	2.5	69	3.76	3.0	81
9	SW 8-46-25-W4	1957	3.24	2.3	72	4.75	4.5	95
42	SW 7-33- 3-W5	1957	3.10	1.0	32	4.14	1.4	33
54	SW36-39- 4-W5	1957	2.83	0.6	22	2.76	1.2	44
53	SW33-38-28-W4	1957	3.56	1.4	40	4.24	3.8	90
24	NE32-40- 2-W5	1957	3.24	1.8	57	3.63	2.6	70
32	SE16-39-28-W4	1957	3.78	2.0	53	3.74	2.8	75
30	NW33-33- 3-W5	1957	2.74	0.9	32	3.02	1.4	46
68	NE32-40- 2-W5	1958*	2.79	1.2	45	3.00	1.1	38
142	SE27-41-28-W4	1958	3.26	2.1	64	3.30	1.4	42
97	NW18-40-25-W4	1958	2.66	1.2	46	2.76	1.6	56
91	NE24-40-26-W4	1958	2.64	1.1	43	3.20	2.2	69
94	SW23-46- 4-W5	1958	3.46	0.9	25	4.24	1.3	30
104	NE33-44- 3-W5	1958	2.82	2.1	74	2.98	1.7	56
117	NE 6-39- 3-W5	1958	2.78	1.0	36	3.23	0.6	19
123	NE35-41-28-W4	1958	2.99	1.4	45	2.66	1.7	63
154	NW20-41-23-W4	1958	2.38	1.4	58	2.72	1.3	47
151	NE20-41-27-W4	1958	3.04	2.2	73	3.30	1.3	40

*Residual effect of sulfur applied in spring 1957.

Table 6 - Effect of sulfur supply on the amide nitrogen and total nitrogen content of Alsike clover

Test No.	Location	Year of Test	No sulfur applied			20 lb./ac. sulfur applied		
			% total N	Amide N		% total N	Amide N	
				mgm./gm. plant material	mgm./gm. total N		mgm./gm. plant material	mgm./gm. total N
			Non-sulfur deficient test areas					
69	SE 1-42-28-W5	1958	2.86	1.7	58	3.31	1.7	53
82	NW12-44- 3-W5	1958	2.84	0.9	33	3.32	1.2	38
90	NW27-38-28-W4	1958	2.65	0.3	10	2.68	0.8	29
132	SE32-41- 3-W5	1958	3.03	1.8	59			
133	NE35-44- 4-W5	1958	2.64	0.6	23	2.79	1.1	40
150	SE20-39- 3-W5	1958	2.91	1.1	38	3.34	1.0	31
			Sulfur deficient test areas					
34	NE19-40- 6-W5	1957	2.28	1.0	46	3.00	1.7	58
38	NE 2-37- 5-W5	1957	1.83	1.2	63	2.08	0.8	38
60	NW34-39- 5-W5	1957	1.83	1.0	56	2.60	2.7	103
61	SW13-40- 3-W5	1957	1.90	1.0	52	2.04	0.8	39
62	NW34-39- 5-W5	1957	1.30	0.8	60	2.13	1.5	69
80	NE21-40-25-W4	1958	2.50	1.1	46	3.08	1.3	43
81	SE 8-41-27-W4	1958	2.46	1.5	61	2.89	1.7	58
88	SW15-46- 5-W4	1958	2.37	1.1	45			
103	NE19-40- 6-W5	1958	2.82	1.0	35	3.36	1.1	32
116	SW32-37- 4-W5	1958	2.62	2.0	77	3.48	1.6	47
120	NW26-44- 4-W5	1958	2.73	1.2	45	3.78	1.2	31
129	NW33-39- 5-W5	1958	2.89	0.9	32	4.03	1.3	33
156	SE24-39- 6-W5	1958	2.64	0.8	29	3.18	0.8	26
174	NE33-44- 3-W5	1958	2.39	1.4	58	3.54	2.0	56

Table 7 - Effect of sulfur supply on the amide nitrogen and total nitrogen content of red clover

Test No.	Location	Year of Test	No sulfur applied			20 lb./ac. sulfur applied		
			% total N	Amide N		% total N	Amide N	
				mgm./gm. plant material	mgm./gm. total N		mgm./gm. plant material	mgm./gm. total N
			Non-sulfur deficient test areas					
73	SE 3-44- 3-W5	1958	3.17	0.6	20	3.33	1.1	34
92	SE30-39- 2-W5	1958	3.20	0.4	11	3.11	0.8	24
115	SE34-43- 2-W5	1958	2.86	0.7	26	2.85	0.8	28
130	NE24-44- 4-W5	1958	2.74	1.0	37	4.02	1.0	24
134	NW11-38- 2-W5	1958	2.39	0.4	18	2.79	0.3	11
149	NW29-45- 6-W5	1958	3.74	1.0	26	3.61	1.3	36
159	NE30-43- 2-W5	1958	3.50	1.1	32	3.20	1.0	32
160	SW20-41- 2-W5	1958	3.48	1.0	28	3.40	0.9	26
163	SW33-45- 7-W5	1958	3.24	0.7	21	3.24	0.9	27
			Sulfur deficient test areas					
1	SE22-35- 5-W5	1957	2.08	1.0	50	2.34	1.9	81
17	SW29-36- 5-W5	1957	1.79	2.0	113	1.66	1.5	89
18	NE19-38- 5-W5	1957	1.96	1.6	81	2.24	2.0	87
20	SE25-33- 5-W5	1957	2.10	1.7	82	2.44	1.7	70
36	NW20-41- 2-W5	1957	1.61	1.0	64	1.84	1.0	55
57	NE19-38- 5-W5	1957	1.49	1.3	86	1.39	0.9	63
58	NW 5-36- 6-W5	1957	2.01	2.0	98	2.04	2.0	98
74	NE22-46- 4-W5	1958	2.25	0.8	37	2.94	0.5	16
75	NW36-40- 6-W5	1958	1.92	6.2	323	2.42	0.5	19
78	SE 3-44- 4-W5	1958	2.78	0.9	32	4.10	1.5	37
87	SE13-43- 3-W5	1958	2.13	0.2	12	3.42	0.5	14
93	NE35-43- 3-W5	1958	2.66	0.5	20	3.22	1.0	32
107	NW31-40- 2-W5	1958	2.84	1.0	36	3.50	1.0	28
114	SE 2-45- 4-W5	1958	2.50	0.8	32	2.77	0.5	17
122	NW22-46- 4-W5	1958	2.36	0.7	29	3.14	0.5	15
155*	SE 2-38- 5-W5	1958	2.66	1.7	65	2.62	1.2	48
161	SW 1-41- 3-W5	1958	2.50	1.0	39	3.82	1.9	49

*Sulfur applied in spring 1957

Table 8 - Total nitrogen content of legumes as affected by seasonal conditions and available sulfur supply

Legume	Sulfur status of soils	Year of tests	No.of tests	% total nitrogen	
				No sulfur applied	Sulfur applied
Alfalfa	Non-deficient	1958	11	2.88	3.01
	deficient	1958	10	2.88	3.14
	deficient	1957	8	3.26	3.76
Alsike	Non-deficient	1958	5	2.78	3.09
	deficient	1958	8	2.63	3.42
	deficient	1957	5	1.83	2.37
Red Clover	Non-deficient	1958	9	3.15	3.28
	deficient	1958	10	2.46	3.20
	deficient	1957	7	1.86	1.99

Soil samples

Thirty-four soil samples, representing both sulfur deficient and non-sulfur deficient test areas, were selected for study. The data for sulfate extractable with 0.001 N hydrochloric acid before and after two weeks' incubation and the weights of Aspergillus niger (M) micelia after five days' incubation are given in Table 9.

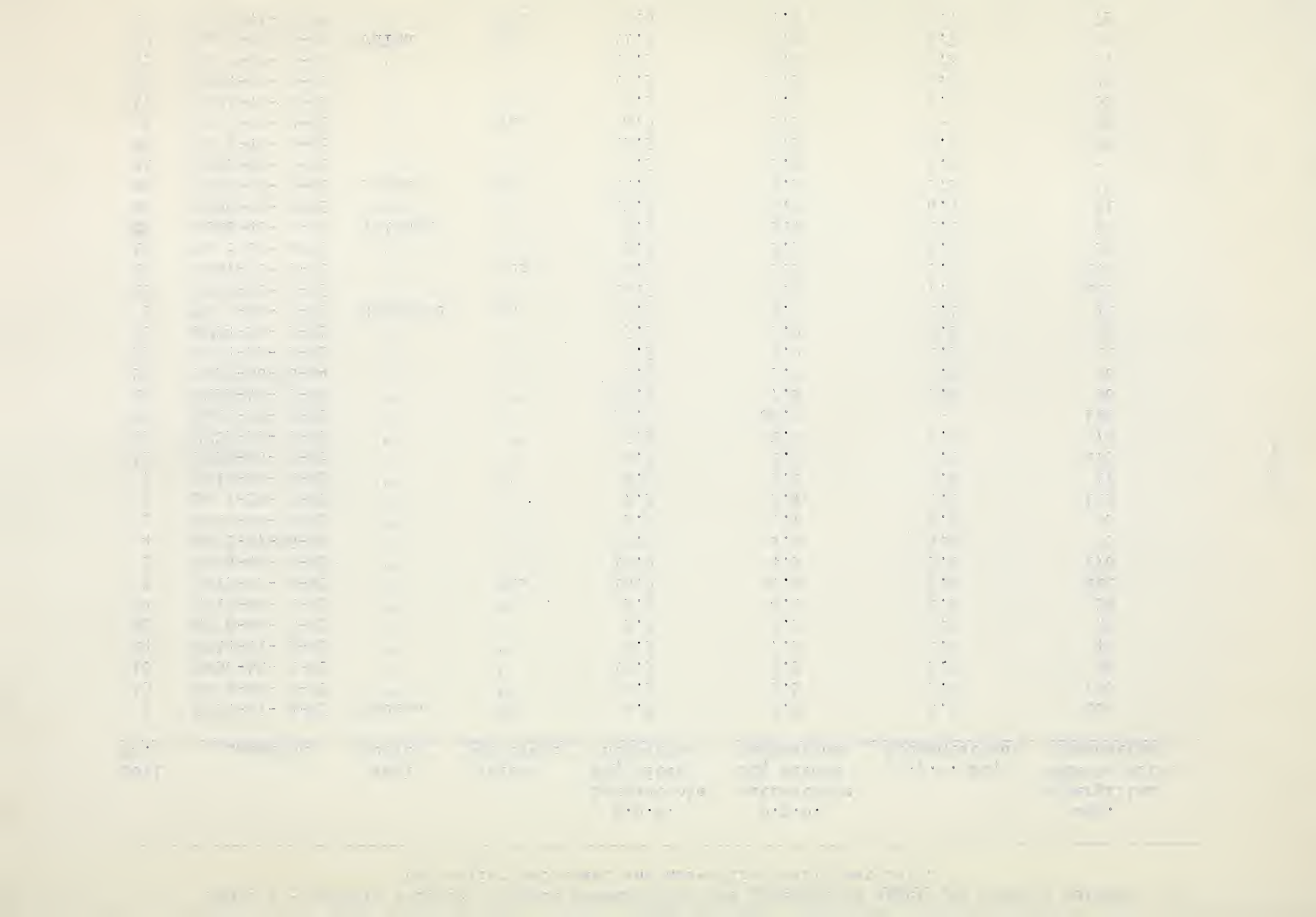
Neither extractable sulfate nor sulfate accumulation bears any relationship to observed sulfur deficiencies. For example, one of the most deficient soils has the highest extractable sulfate content.

The data for the Aspergillus niger (M) test represent the actual weights in milligrams of the micelia produced. For comparison purposes, nutrient solutions containing known amounts of sulfate were prepared and incubated but the mycelia were lost through faulty techniques in washing and drying. The data do not indicate any definite differences between sulfur deficient and non-sulfur deficient soils even within the same soil

series. Although this agrees with visual estimates made on the growth, the techniques used in growing, harvesting and drying the mycelia were not entirely satisfactory and too much emphasis should not be placed on these particular data.

Table 9 - Soluble sulfate, sulfate accumulation and Aspergillus niger (M) micelia weights for sulfur deficient and non-sulfur deficient soils

Soil No.	Location	Soil Series	Sulfur Deficient	p.p.m. Extractable SO_4 after Incubation	p.p.m. Extractable SO_4 before Incubation	p.p.m. SO_4 Accumulation	mgm. Aspergillus micelia after Incubation
1	SW34-37- 4-W5	Breton	No	6.0	3.9	2.1	189
15	NW 6-44- 2-W5	"	"	10.5	5.5	5.0	116
18	NW36-42- 1-W5	"	"	10.5	5.5	5.0	69
24	NW14-41- 3-W5	"	"	9.5	7.5	2.0	47
33	NW 6-42- 2-W5	"	"	9.0	5.5	3.5	71
14	NW12-43- 1-W5	"	"	9.0	6.1	2.9	30
2	NW15-43- 3-W5	"	Yes	10.0	4.0	6.0	222
3	NE28-46- 3-W5	"	"	10.0	4.9	5.1	176
4	NE 5-37-26-W4	"	"	10.0	6.0	4.0	5
5	NE19-40- 6-W5	"	"	5.0	4.1	0.9	89
6	SW 7-33- 3-W5	"	"	7.5	3.4	4.1	113
7	SE16-39- 1-W5	"	"	8.0	5.2	2.8	17
11	NW32-46- 3-W5	"	"	6.0	2.9	3.1	275
13	SW29-36- 6-W5	"	"	5.5	2.6	2.9	70
19	SW11-39- 5-W5	"	"	32.0	60.0	-	116
21	NE35-43- 3-W5	"	"	15.5	7.6	7.9	86
30	SW33-38-28-W4	"	"	9.0	5.1	3.9	6
35	NW34-39- 5-W5	"	"	7.5	6.0	1.5	22
36	NW34-39- 5-W5	"	"	5.5	5.0	0.5	49
9	SW 3-38- 7-W5	Caroline	No	6.5	4.4	2.1	72
29	NW31-36- 4-W5	"	"	14.5	7.0	7.5	123
12	NE27-35- 5-W5	"	Yes	6.5	3.3	3.2	140
16	NW 5-36- 6-W5	"	"	9.5	3.5	6.0	30
22	SE28-43- 2-W5	Tollman	"	4.5	3.4	1.1	20
26	NW36-40- 6-W5	"	"	12.5	5.9	6.6	91
28	NW24-39- 3-W5	Rimbey	No	12.0	8.5	3.5	14
31	SW35-43- 3-W5	"	"	7.5	5.8	1.7	-
34	SW 5-42- 3-W5	"	"	13.5	2.9	10.6	94
8	NE31-40- 2-W5	"	Yes	8.0	9.5	-	34
17	NE31-40- 2-W5	"	"	6.5	4.2	2.3	53
20	SE24-39- 6-W5	"	"	10.5	10.5	0.0	23
25	NE 6-39- 3-W5	"	"	10.5	8.2	2.3	37
23	SE 4-42- 1-W5	Falun	No	11.5	6.8	4.7	24
10	NE20-41-27-W4	"	Yes	9.0	4.6	4.4	75



CONCLUSIONS

The data obtained in this study suggest the following conclusions:

1. Soil tests for extractable sulfate, sulfate accumulation on incubation and the growth of Aspergillus niger (M) were not satisfactory tests for differentiating between soils on which legumes would not respond to sulfur fertilization and soils on which legumes would respond to sulfur fertilization.

2. The amide nitrogen content of alfalfa, alsike clover and red clover did not vary consistently with the level of sulfur nutrition of the plants.

3. The extractable sulfate content of alfalfa and alsike clover proved to be a reliable guide to the need for sulfur fertilization. This measure was accurate for 89 per cent of the tests from which alfalfa was analysed and 95 per cent of the tests from which alsike clover was analysed. The extractable sulfate content of red clover was not satisfactory.

4. The extractable sulfur content of the three legume crops was a reliable criterion for determining the need for sulfur fertilization. This analysis was an accurate measure for 89 per cent of the alfalfa samples, 100 per cent of the alsike clover samples and 88 per cent of the red clover samples.

5. Tentative critical levels of extractable sulfate and extractable sulfur for the three legume crops were found to be:

	Extractable sulfate %	Extractable sulfur %
Alfalfa	0.09	0.21
Alsike clover	0.04	0.07
Red clover	--	0.08

APPENDICES

I. Tests for ammonia release in unfertilized plant material when analysed for amide nitrogen by the distillation method of Varner et al. (68)

Sample No.	Legume	From sulfur deficient soil	Ammonia distilled off	Mgm. N/gm. plant material	Diff.
1	Red clover	yes	yes	1.11	
2	" "	"	no	1.04	-0.07
3	" "	no	yes	0.97	
4	" "	"	no	0.97	0.00
5	Alsike clover	yes	yes	1.53	
6	" "	"	no	1.39	-0.14
7	" "	no	yes	1.87	
8	" "	"	no	1.94	+0.07
9	Alfalfa	yes	yes	1.60	
10	"	"	no	1.60	0.00
11	"	no	yes	1.53	
12	"	"	no	1.46	-0.07

II. Results of tests for the recovery of amide nitrogen in asparagine and glutamine when analysed by the method of Varner et al. (68)

Sample No.	Amide added	Millimoles Amide Added	Recovered	% Recovery
1	Asparagine	0.0146	0.0136	93
2	"	"	0.0137	94
3	"	"	0.0133	91
4	Glutamine	0.0096	0.0092	96
5	"	"	0.0087	91
6	"	"	0.0093	97

III. Tests for the comparison of three sulfate extractants for soils and for two procedures for the clarification of extracts

Beaker No.	Soil No.[1]	Extractant	Method of clarification	p.p.m. SO_4 Duplicates 1	2
1	1	0.001 N HCl	Charcoal	32	38
2	1	0.1 N HCl	"	41	43
3	1	70% EtOH	"	2	2
4	2	0.001 N HCl	"	5	6
5	2	0.1 N HCl	"	2	2
6	2	70% EtOH	"	1	3
7	3	0.001 N HCl	"	5	5
8	3	0.01 N HCl	"	1	1
9	3	70% EtOH	"	1	1
10	4	0.001 N HCl	"	9	11
11	4	0.1 N HCl	"	1	2
12	4	70% EtOH	"	1	1
13	5	0.001 N HCl	HNO_3 - $HClO_4$	8.3	
14	5	0.001 N HCl	Charcoal	5.4	
15	5	70% EtOH	HNO_3 - $HClO_4$	1.9	
16	5	70% EtOH	Charcoal	1.4	
17	Stnd. SO_4	--	Charcoal	20	
18	Stnd. SO_4	--	--	20	

[1] Soils 2 and 3 sulfur deficient, others non-sulfur deficient.

IV. The extractable sulfate and extractable sulfur content of leaf and leaf plus stem samples of alfalfa and red clover

Material	Extractable sulfate %	Extractable sulfur %[1]
Alfalfa leaves	0.406	0.580
Alfalfa leaves and stems	0.350	0.468
Red clover leaves	0.015	0.036
Red clover leaves and stems	0.018	0.039

[1] Expressed as percentage sulfate.

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